METHOPRENE

A review of the impacts of the insect growth regulator methoprene on non-target aquatic organisms in fish bearing waters (Ver. 2.0)

For the Massachusetts Pesticide Board Subcommittee
By Steven Antunes-Kenyon and Gerard Kennedy.
Massachusetts Pesticide Bureau, Department of Food and Agriculture,
251 Causeway Street, Boston, MA 02114-2151
www.massdfa.org
August, 2001

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EXECUTIVE SUMMARY

This document reviews the environmental impacts of the insect growth regulator methoprene, when used for midge and mosquito control, on non-target aquatic species in fish bearing waters. Increased reports of specific malformations in amphibians over the past decade, confusing regulatory decisions at the state and federal level regarding the use of methoprene, and the general public concerns which have been expressed about methoprene use led the Massachusetts Pesticide Board Subcommittee to direct the Massachusetts Pesticide Bureau to conduct this review.

Methoprene is an insect growth regulator, which is highly effective as a control agent for mosquitoes. It interferes with maturation and reproduction in insects by mimicking the activity of natural juvenile insect hormone. Chemically it is considered to be a member of the terpenoid family of chemicals. Technical methoprene is soluble in water at 1.39 ppm and very soluble in common organic solvents.

Methoprene, for use in mosquito and midge control is sold under the trade name "Altosid®". Wellmark International manufactures and distributes the Altosid® line. Among the commercially available Altosid® products are slow release formulations such as briquets, which release the active ingredient continually when wet as they erode over periods ranging from 21 days to 150 days. Altosid® is widely used in Massachusetts as a mosquito larvicide in municipal West Nile Virus prevention strategies.

Methoprene is rapidly degraded under field conditions. The biological activity of aqueous solutions of methoprene is affected by sunlight, temperature, and microbial action. The metabolism of methoprene by aquatic microorganisms is extensive. The most abundant breakdown product in aqueous solution is methoxycitronellal (9%). While methoprene degrades rapidly in the field, the persistence in water is determined, ultimately however, by the formulation and method of application. The XR briquet releases small amounts of methoprene as it degrades over a period of 150 days. While the briquet has been shown to physically persist in water for eighteen months, no data is available to indicate biological activity for this time period.

Although methoprene is most toxic to insects of the order Diptera, it is also toxic to a range of insects from 12 orders, including Diptera, Lepidoptera and Coleoptera. In all cases reviewed, mosquitoes and midges show the greatest susceptibility to methoprene. Toxicological studies reviewed observed variable susceptibilities by non-target aquatic invertebrates to methoprene. Short-term toxicity studies on insects indicate that most non-target insects, including those predacious to mosquitoes, are not likely to be adversely impacted by labeled applications of methoprene. While a number of studies on the grass shrimp and daphnia magna raise concerns, the majority of studies reviewed suggest that there is not likely to be an impact on crustaceans at expected environmental concentrations. However, research is ongoing in this area, particularly on lobsters.

The United States Environmental Protection Agency (EPA) has registered all commercially available Altosid® formulations of methoprene for use in fish bearing waters. However, the 1991 EPA RED factsheet continued to state until recently, that the pesticide is acutely toxic to estuarine and marine invertebrates. The factsheet was updated in June 2001 to remove this statement.

At the state regulatory level, however, inconsistencies remain regarding the use of methoprene. New York, due to concerns about the teratogenicity of methoprene breakdown products,

continues to prohibit the application of sustained-release formulations to fish bearing waters. Maryland limits the uses of methoprene on a case-by-case basis through its pesticide aquatic application permitting process. While Altosid® is registered for use in fish bearing waters in all other states, a number of states have placed limitations on the application of methoprene products, along with dozens of other pesticides, to waters containing endangered species.

The overall findings of the review are:

- We have found no evidence to suggest that the labeled application of methoprene for mosquito and midge control will lead to amphibian malformations.

- Studies reviewed observed variable susceptibilities of crustaceans to methoprene. At this time, it is difficult to draw final conclusions regarding the safety of methoprene for crustaceans until further research is completed and available for review. The weight of evidence reviewed, however, suggests that impacts upon crustraceans are not likely at expected environmental concentrations.

- Because the half life of methoprene is quite short , the use of the liquid larvicide is unlikely to create any adverse impacts. Possible exceptions are repeated applications, or the use of methoprene slow release formulations in shallow, poorly flushed waters. The data gap for chronic exposure to small quantities of methoprene over the long term, particularly in a poorly flushed medium, prevents conclusions from being drawn about the long term effects of the 150 day slow release formulation.

- While some impact on non-target organisms (especially in aquatic communities) could be expected, the effects of methoprene application would be less harmful than those caused by most mosquitocidal pesticides. Methoprene has longer persistence than *Bti* after application, but also causes greater impact on non-target organisms. Despite this, there is no indication in the literature of permanent disruption to ecosystems after methoprene application.

1. INTRODUCTION

The purpose of this report is to review and evaluate the environmental impacts of the insect growth regulator methoprene[1], when used for midge and mosquito control, on non-target aquatic species in fish bearing waters. The Massachusetts Pesticide Bureau on behalf of the Massachusetts Pesticide Board Subcommittee is conducting the review. The subcommittee requested the review in response to a letter from an environmental group[2] which asks that the subcommittee "revoke the use of Methoprene based pesticides in bodies of water containing fish and shell fish." Among the claims in the letter are that: "Methoprene when exposed to sun-light and water breaks down into retinoids that causes deformities in frogs, fish and other aquatic invertebrates"; "ten states have banned the use of Methoprene in bodies of water containing fish"; and the material safety data sheet on two methoprene based pesticides state that it is toxic to aquatic invertebrates.

While several of the assertions in the letter are inaccurate, there *have* been increased reports of specific malformations in amphibians over the past decade that have raised public concerns about the possible causes. Observed deformities include missing limbs and digits and central nervous system malformations (Ankley, 1998). Speculation as to the causes has focused on ultraviolet light, a parasitic flatworm and biochemicals, such as retinoids, methoprene and derivatives of methoprene. Methoprene has also been implicated as a causative agent of a lobster die-off in Long Island Sound in 1999.

Regulatory decisions at the state and federal level have also muddied the waters regarding the issue of methoprene use. While methoprene is registered for use in all 50 states, two slow release formulations are presently prohibited from use in fish bearing waters in New York. Maryland places conditions on the use of methoprene on a case-by-case basis. Furthermore a number of states have placed limitations on the use of methoprene (along with dozens of other commonly used pesticides) in waters containing endangered species. Additional confusion has been added due to conflicting information between the federal label provisions for Altosid® use and 1991 EPA methoprene factsheet[3], The factsheet stated that "methoprene is highly acutely toxic to estuarine invertebrates", while the label contains no such information. The factsheet was updated in June 2001 to reflect the fact that concerns about toxicity to estuarine invertebrates have been alleviated as a result of the submission of studies which indicate minimal chronic risks[4].

Given the attention methoprene has received as a potential causative agent of amphibian deformities, the apparent inconsistencies among federal and state regulators regarding its use patterns, and the general public concerns which have been expressed regarding its use, it is important for the Pesticide Board Subcommittee to have a clear understanding of the issues. This review of methoprene will assist the subcommittee in its decision making by reviewing the scientific literature as it pertains to the toxicity of methoprene to non-target aquatic organisms and to the predicted fate and transport of methoprene in aquatic systems. It also presents:

- an analysis of the conflicting information from EPA regarding methoprene use,
- a discussion of how methoprene is regulated in other states, and
- a brief comparison of alternative methods of control.

[1] Methoprene for mosquito or midge control purposes is sold under the trade name ALTOSID®.
[2] Preserve Our Pond. December 15, 2000. Letter to the Director of Regulatory Services
[3] 1991 Environmental Protection Agency Reregistration Eligibility Document (RED)
[4] 2001 Environmental Protection Agency Reregistration Eligibility Document (RED)

2. BACKGROUND

Methoprene is an insect growth regulator which is highly effective as a control agent for mosquitoes. It interferes with maturation and reproduction in insects by mimicking the activity of natural juvenile insect hormone. Chemically it is considered to be a terpenoid. The most notable commercially available formulations are the slow release pellet and briquet products which release methoprene continually as they erode over periods up to 150 days.

Methoprene is an insect growth regulator that has been registered as a general use pesticide by EPA since 1975. Methoprene has no significant adverse toxicological effects in any human health effects screening studies and has been classified as a slightly to practically nontoxic compound by EPA, which ranks it in "toxicity class IV" (EPA, 1991). It is considered to be a biochemical pesticide because, rather than controlling insects through direct toxicity, it disrupts the insect's lifecycle and prevents it from reaching maturity and reproducing. Because it is effective in controlling the larval stage of insects, it is widely used as a larvicide (EXTOXNET, 1996). Methoprene has been extensively tested against *Aedes* mosquitoes and shown to be highly effective, both in fresh and salt water (Glare, 1999). In aquatic areas, it is used to control mosquitoes, and several types of flies, moths, beetles and fleas. It is also registered for use on a number of foods including meat, milk, eggs, mushrooms, peanuts, rice, and cereals.

As a potent insect growth regulator (IGR), methoprene interferes with maturation and reproduction in insects by mimicking the activity of natural juvenile insect hormone [5](Wright, 1976). During development, insects undergo changes at specific times, for example pupation, which are mediated by endogenous hormones, such as juvenile insect hormone. Juvenile insect hormone, expressed at certain specific times, leads to metamorphosis. However, if present at other times, the presence of juvenile insect hormone leads to suppression of adult characteristics. These abnormalities are observed during molt into the pupae or adult stages of growth. For example the feet of treated mosquito may be stuck to the molted skin or covering (exuvia) of the pupae causing failure of the newly formed adult to emerge from the water. Insects and crustacea whose metamorphosis is regulated by a juvenile insect hormone and a molting hormone could be sensitive to methoprene during development. Animals lacking juvenile insect hormone should not be sensitive. The activity of methoprene should not be confused with that of chitin inhibitors[6] such as diflubenzuron.

Insect growth regulators, such as methoprene, interfere with insect development causing death or reproductive failure at a specific time in the life cycle, usually not the stage treated. Glare states that: "the extent and character of the response varies between insects, but generally it is the last instars of the larval or nymph form, or pupae, which are most affected" (Glare, 1999). For example, mosquito larvae are the target stage for methoprene, but the effect is not seen until lack of adult emergence.

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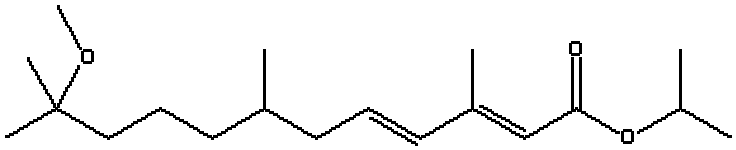
[5] **juvenile hormone,** also called ECDYSONE, a hormone in insects, secreted by glands near the brain, that controls the retention of juvenile characters in larval stages. The hormone affects the process of molting, the periodic shedding of the outer skeleton during development, and in adults it is necessary for normal egg production in females *(Enclycopaedia Britannica Online)*

[6] Chitin-inhibitors such as Dimilin (diflubenzuron) have a much broader effect on non-target organisms (Glare, 1999).

Chemical and Physical Properties

S- Methoprene (Isopropyl (2E,4E)-11- Methoxy- 3,7,11- trimethyl-2,4-dodecadienoate) is a pale yellow liquid with a fruity odor. Its chemical structure is typical of the family of chemicals known as terpenoids:

Figure One: Isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate;



Source: www.chemfinder.com

Many terpenoids are naturally occurring and include vitamin A, camphors, citronell and limonene. Table One shows some of the basic physical and chemical properties of methoprene. The chemical structure of methoprene is very similar to natural juvenile insect hormone. Both chemicals are long chain esters containing only carbon, hydrogen and oxygen and have similar molecular weights.

Table One: Physical and Chemical Properties of S-Methoprene. (The Pesticide Manual, 2000 and Wellmark International)

Molecular Weight	310.50	***Vapor Pressure*** *(RS- Methoprene)*	2.37 X 10^{-5} mm Hg at 25° C
Molecular Formula	C_{19} H_{34} O_3	***Specific Gravity***	0.921 (25° C)
Form	Pale yellow liquid with a fruity odor	***Solubility In water***	1.39 ppm
Boiling Point	> 262 25° C ° C	***Stability in water***	Stable in water; sensitive to UV light

Commercial Availability

Methoprene, for use in mosquito and midge control is sold under the trade name "Altosid®". Wellmark International manufactures and distributes the Altosid® line. Among the commercially available Altosid® products are slow release formulations such as briquets, which release the active ingredient continually when wet as they erode over periods ranging from 21 days to 150 days. Application rates vary depending on the type of habitat, water depth and water quality. Maximum application rates are shown in Table Two. All of the Altosid® products are formulated with the resolved S-methoprene enantiomer except for the 30 day briquet which contains both the R and the S enantiomers.

Table Two: Altosid® Product Line

ALTOSID® PRODUCT	EPA #	% ACTIVE INGREDIENT[7]	APPLICATION RATE	RELEASE PERIOD
LIQUID LARVICIDE	2724-392	5%	3 to 4 fl.oz/ A	5 – 7 days
LIQUID LARVICIDE	2724-446	20 %	0.75 – 1 fl oz/ A	5- 7 days
ALTOSID® SBG	2724-489	0.2%	5-10 lbs per acre	5- 10 days
BRIQUETS *	2724-375	8.62%	1 to 4	30 days
BRIQUET XR*	2724-421	2.1%	1 briquet/ 100 sq ft to 1 briquet/ 200 sq.ft for non flow shallow (<2 feet) areas.	150 days
PELLETS*	2724-448	4.25 %	2.5 – 10 lbs per acre	30 days
GRANULES XR*	2724-451	1.5 %	5- 10 lbs per acre	21 days

***SLOW RELEASE SOLIDS**

[7] Information on the "inert" ingredients is proprietary and not available.

3. TOXICOLOGICAL PROFILE
(a) Amphibians, (b) Insects, (c) Crustaceans, (d) Oysters, (e) Fish.

(a) Amphibians

The global decline in amphibian populations and recent increases in reported amphibian malformations has led to a surge in amphibian research and the development of more than one plausible theory for its explanation. The severity and types of malformations found in nature vary widely; however, a great majority of them are found in the hindlimbs and include extra (supernumary) limbs, skin webbings, and missing legs (Ankley, 1998). The theory that certain breakdown products of methoprene might mimic the action of retinoids and cause malformations in amphibian populations is partially supported by research that discovered how methoprenic acid (t-MA) can stimulate gene transcription in vertebrates. In fact these findings appear to build on the studies showing an increase in limb deformities from methoprene treated mouse embryos (Harmon, 1995).

Upon close examination of a number of studies related to these issues, it appears that the theory implicating methoprene or its naturally occurring breakdown products and their alleged affects on the retinoic acid pathway of development is highly questionable and largely without merit.

- **Methoprenic acid (t-MA) is not a naturally occurring compound (Harmon, 1995)**
- **The lowest concentrations of sunlight exposed methoprene shown to cause any malformation was 7.5 µL/L (ppm), which is some 1,700 times the level found under typical application rates of Altosid® (La Clair, 1998)**
- **Studies by La Clair were unable to produce the most commonly seen malformations, such as supernumary limbs, skin webbings, and missing legs. Severe eye malformations and other cranial and facial defects were the primary observations in La Clair's work (La Clair, 1998)**
- **There is greater evidence that recent increases in malformed amphibians may be explained by increases in exposure to unsafe levels of UV light and increases in the rate of amphibians infection by parasitic trematodes.**

Methoprene has received considerable attention as a possible causative agent of the increase in amphibian malformations over the past decade (Ankley et al 1998). The growing number of reported malformations are primarily from the amphibian **order Anura** (frogs and toads); however, a number of reports also include the **order Caudata** (salamanders). A high prevalence of hindlimb deformities has been recorded in wild-caught green frogs (*Rana clamitans*), northern leopard frogs (Rana pipiens), American toads (Bufo americanus) and bullfrogs (Rana catesbeiana) from agricultural lands (Ouellet, 1997). Other amphibians species exhibiting gross malformations include gray tree frogs (*Hyla versicolor*), mink frogs (*Rana septentrionalis*), wood frogs (*Rana sylvatica*), spring peepers (*Hyla crucifer*), Pacific tree frogs (*Hyla regilla*), long toed salamanders (*Ambystoma macrodactylum*), and spotted salamanders (*Ambystoma maculatum*) (La Clair, 1998). Based on these and other anecdotal reports, most researchers consider the malformations to be at levels that are above "normal".

Some of the common external abnormalities observed in the field include the following:

- Extra limbs (supernumary limbs or polymelia)
- Missing limbs or limb segments (ectromelia)
- Limbs located in an unusual place (ectopic limbs)
- Extra digits or toes (polydactyly)
- Missing or misplaced eyes
- Skin webbing (cutaneous fusion)
- Missing part or all of one or more digits or toes (ectrodactyly)

Due to the aquatic developmental stages of amphibians and their associated gill and transdermal respiration mechanisms, potential exposure to and absorption of xenobiotics, such as juvenile hormone, may occur at the embryonic, larval and metamorphic stages. Some researchers are looking at the retinoic acid pathway as a target of teratogenic activity.

Retinoids, such as retinoic acid, are biochemical metabolites of Vitamin A, which regulate gene expression and appear to play a role in vertebrate limb development (Thaller, 1993). The retinoic acids modulate gene expression by binding to nuclear retinoic acid receptors (RAR) and retinoid X receptors (RXR), forming complexes which then act on DNA sequences known as hormone response elements (Harmon, 1995). In excess, retinoids can be teratogenic, causing serious birth defects in humans. All-*trans*-retinoic acid (t-RA) is known to have profound effects on cellular differentiation, pattern formation, and embryonic development (Yang, 1991). La Clair reports that certain concentrations of t-RA cause nervous system and craniofacial malformations [8]. According to Sessions *et.al.* some of the more commonly seen malformations in the field, supernumary limbs, can also be experimentally induced in tadpoles by treatment with retinoic acid (Sessions, 1999).

Methoprene and its derivatives share structural and behavioral similarities with the retinoids. There is evidence that *trans*-S-methoprenic acid, a laboratory breakdown product of methoprene, can mimic the behavior of t-RA. Trans-S-methoprenic acid (t-MA) has been shown to stimulate gene transcription in vertebrates by binding to a cellular retinoid receptor. The researchers concluded that the discovery of t-MA activity may explain the reported teratogenic effects of high doses of methoprene that have been observed during mouse embryogenesis. Effects observed from these embryogenesis studies included limb deformities similar to the effects of retinoids (Harmon, 1995).

La Clair *et.al.* report that, at extremely high levels, methoprenic acid was found to dramatically interfere with normal amphibian development. Exposure to methoprene causes little mortality and malformation to *Xenopus laevis* embryos at concentrations below 15 µL/L (ppm). However, a mixture of methoprene and its photodegradation products caused malformations at concentrations of 7.5 µL/L[9] (ppm), a concentration some 1,700 times the level found under typical applications. Results of additional experiments using chemically synthesized pure t-MA show significant levels of deformities at 2.5 µL/L (ppm) and complete malformation at 15 µL/L (ppm). The photolysis of t-MA showed increased malformations at 7.5 µL/L (ppm) and obvious mortality at 10 µL/L (ppm)

[8] 96 hour whole embryo developmental toxicity study: Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX)
[9] 1 µL/L is equal to 1 part per million (ppm). The unit µL/L is a ppm weight/weight description of concentration based on the followng: ppm (w/w) = (g of analyte in sample / g of sample) x 10^6.

There are several flaws in La Clair's work. The concentrations of test compounds used in his studies do not remotely mirror those found under typical conditions. The lowest dose of sunlight exposed methoprene show to cause any malformation was 7.5 µL/L (ppm). As La Clair points out in his discussion, when correctly applied to a 1 hectare (he) (2.49 acres) pond with an average depth of 0.25 m, the concentration of S-methoprene lies between 0.0044 and 0.0060 µL/L (ppm). Furthermore, although the theoretical hydrolysis product t-MA was shown to be of higher developmental toxicity to *Xenopus laevis* than its parent compound, it is not found in nature and cannot be studied outside of the laboratory. Lastly, the studies by La Clair were unable to produce the most commonly seen malformations, such as supernumary limbs, skin webbings, and missing legs. Severe eye malformations and other cranial and facial defects were the primary observations in La Clair's work.

In a U.S. EPA study presented as a poster abstract at the 2001 Society of Toxicology Annual Meeting, Degitz ***et.al.*** describe their findings of the developmental toxicity of methoprene and its degradation products in the African clawed frog (***Xenopus laevis).*** In 96-hour *in vivo* assays (FETAX) methoprene, methoprene acid, methoprene epoxide, 7-methoxycitronellal and 7-methoxycitronellic acid were tested for their ability to cause malformations at concentrations ranging from 0.1 µl/L (ppm) to 30 µl/L (ppm).

Results from the above study demonstrate the potential for methoprene acid to cause craniofacial malformations at exceedingly high concentrations [$\geq$1.25 µl/L (ppm)]. Methoprene epoxide and 7-methoxycitronellal also induced malformations but only at concentrations $\geq$5 µl/L (ppm). The authors conclude that methoprene and its metabolites are not potent developmental toxicants to the African clawed frog (***Xenopus laevis).*** They further found that methoprene concentrations in their experiments were 3-orders of magnitude higher than expected environmental concentrations based on typical field application rates of Altosid® (0.01 ppm) and that concerns or methoprene-mediated developmental toxicity may be unwarranted.

Another study looked at the effects of ultraviolet light and methoprene on the survival and development of embryos of Northern leopard frogs (*Rana pipiens*). Ankely ***et. al.*** exposed newly fertilized eggs of Northern leopard frogs (*Rana pipiens*) to several different concentrations of methoprene both in the presence and absence of UV light. The concentrations of methoprene ranged from 0 to 488 µg/L (ppb).[10] Methoprene treatment at the four lowest test concentrations did not result in increased mortality or developmental abnormalities in Northern leopard frogs, either in the absence or presence of UV light. However, all of the embryos from the high methoprene treatments were grossly deformed, exhibiting severe axial distortions, as well as craniofacial and abnormalities in the tail or posterior. By day 16 all organisms in high methoprene treatment, both in the absence and presence of UV light, were dead. Interestingly, exposure to the pesticide did not cause any limb malformations, which is the effect that is generally reported in wild amphibian populations. More than half of the frogs which were exposed to UV light for 24 days developed hindlimb malformations, irrespective of whether methoprene was present. These malformations included missing limbs segments and missing or reduced digits. Despite being similar to those seen in deformed frogs in the wild, the UV light did not cause the full range of malformations observed in the field. While the conclusions to the study were uncertain, the researchers did suggest that UV light should be considered as a plausible factor contributing to amphibian malformations in field stations.

[10] Concentration ranges are based on min. and max. from 2 sample days.

Possible links between the increased amphibian infection rates by parasitic trematodes has also been implicated in the surge of observed amphibian malformations. One of the leading papers on amphibian malformations was completed in 1990 and specifically examined one explanation for naturally occurring extra (supernumary) limbs in amphibians. This work has led the investigation of how a parasitic flat worm (trematode) uses amphibians as intermediate hosts. The cercarial larval stage of the trematode attacks amphibians, penetrating the skin to form cysts (metacercariae). The cysts are preferentially localized in the developing hind limb regions of both salamanders and frogs (Sessions, 1990).

One recent study from the literature shows how severe limb abnormalities were induced at high frequencies in Pacific tree frogs (*Hyla regilla*) exposed to cercariae of a trematode parasite (*Ribeiroia sp.*). The abnormalities closely matched those observed at field collection sites, and elevations in parasite density led to an increase in malformation frequency and a decline in the survival of tadpoles (Johnson, 1999).

(b) Insects

Although methoprene is most toxic to insects of the order Diptera, it is also toxic to a range of other insects from 12 orders, including Hemiptera, Lepidoptera and Coleoptera. The lethal dose required to kill common mosquitoes is generally around 1 part per billion (ppb). In all cases reviewed mosquitoes and midges show the greatest susceptibility to methoprene (Miura, 1973 and Lawler, 2000). The short-term toxicity studies reviewed indicate that most non-target insects, including those predacious to mosquitoes, are not likely to be adversely impacted by labeled applications of Altosid®. This finding is due to the fact that expected environmental concentrations are significantly lower than acute toxicity endpoints for most aquatic non-target insects.

In nature, insect predators are an important natural control agent of insect pests such as mosquitoes. Consequently, it is important to evaluate methoprene use for effects against such biological agents.

In a short-term study the effects of ZR-515 [(RS)-methoprene] were tested on a number of non-target insects. A series of five methoprene concentrations were utilized for the laboratory studies. Water boatman *(C. decolor)* and backswimmers *(N. unifasciata)* were maintained in metal barrels treated with 10% ZR-515 and containing 0.1 ppm (RS)-methoprene. The following insects were included in these studies performed in the laboratory, in outdoor artificial containers, and in the field:

- damselfly nymphs *(Argia sp.)*
- dragonfly nymphs *(Orthemis sp.)*
- mayfly nymphs *(Callibactis sp.)*
- water boatman nymphs and adults *(Corisella decolor sp.)*
- backswimmer nymphs and adults *(Notonecta unifasciata sp.)*
- diving beetle adults *(Laccophilus sp.)*
- water scavenger beetle larvae and adults *(Helophorus sp. and Hydrophilus triangularis sp.)*, *(Tropistcrus lateralis sp.)*
- whirligig beetle adults *(Gyrinus punctellus)*
- flower fly larvae *(Xylota sp.)*
- shorefly larvae *(Brachydcutera argentata)*
- midge largae *(Chironomum stigmaterus)*
- mothfly larvae *(Pericoma sp.)*

Results of acute toxicity tests with ZR-515 (technical) performed in the laboratory with non-target predatory insects and mosquitoes are presented below:

Table Three: Risk Quotients for Select Insects (Miura, 1973).

Animal	State of growth	Number of tests	Number Animals per container	Test Duration (hours)	LC_{50} (ppm)	Acute RQ[11] (EEC/LC_{50})[12]
Water boatman *(Corisella decolor sp.)*	Adults	4	10	24-96	1.65	0.006
Backswimmers *(N. unifasciata)*	Nymphs	4	10	24	1.20	0.008
Diving beetles *Laccophilus sp.*	Adults	3	10	48-72	2.00	0.005
Mosquitoes *Ochlerotatus nigromaculis*	Larvae	9	25	96-120	8×10^{-6}	1,250

The maximum expected environmental concentrations of methoprene from labeled application rates is 10 µg/L (ppb) or 0.01 mg/L (ppm). In the above study backswimmers appear to be the most sensitive species with an acute median lethality (LC_{50}) of 1.20 mg/L (ppm). In addition to the fact that the acute toxicity endpoint for the most sensitive species in this study is some 100 times the EEC, the calculated risk quotients for all organisms indicate minimal acute risks of toxicity. This study also helps to illustrate that mosquitoes (*Ochlerotatus nigromaculis)* are significantly more sensitive to methoprene than other non-target insects (Miura, 1973).

Lawler *et.al.* studied the effects of sustained-release methoprene and a combined formulation (Duplex) of liquid methoprene and *Bti* on insects in salt marshes. Although, methoprene concentrations in treated water were not measured, these materials were applied at maximum label application rates. The authors expressed concern for the development of resistance as a result of the use of sustained-release formulations; however, they found no evidence that Altosid® Pellets or the Duplex mixture affected the survival of water boatman[13] *(Trichocorixa reticulata)*. Caged juvenile water boatman matured at the same rates in treated and control sites and there were no apparent malformations. The relative insensitivity of water boatman *(Trichocorixa reticulata)* to methoprene demonstrated in this study supports the above findings by Miura and Takahashi (Lawler, 2000).

Methoprene was reported to have no deleterious effect on backswimmers[14] (*Notonecta unifasciata)* and (*Buenoa scimitar)*, when used to control mosquitoes in California. A different study from California however, showed that repeated applications of 100 µg/L (ppb) methoprene to experimental ponds eliminated larva of the diving beetle[15] (*Laccophilus* sp.). These adverse effects represented a loss of 84% of the predator biomass during one period. Dragon fly and

[11] Risk Quotient (RQ): The estimated environmental concentration / median lethal dose (LC_{50}); The lower the Risk Quotient (RQ) the less risk.
[12] According to Ross *et.al.* 10 µg/L (ppb) or 0.01 mg/L (ppm) is the Expected environmental concentrations from the application of ALTOSID Liquid Larvicide at 4 fluid oz. /acre (293 ml/ha) (Ross, 1994).
[13] The **order Hemiptera** includes aquatic predators known as water boatman from the **family Corixidae**.
[14] The **order Hemiptera** includes aquatic predators known as backswimmers from the **family Notonectidae**.
[15] The **order Coleoptera** includes aquatic predators knows as diving beetles from the **family Dytiscidae**.

damsel fly naiads[16] formed the second major group of predators during the study; these preyed heavily on mosquitoes and ostracods[17] and were not affected by Altosid®. In another study, two larval predators, damselfly naiads *(Enallagma* sp.) and diving beetles appeared not to be affected by the Altosid® applications against mosquitoes in Florida (Glare 1999).

The short-term effects of methoprene and *Bti* on non-target insects were studied by Hershey *et. al.* using temporary pond in Wright County Minnesota, Minnesota. Methoprene was applied as Altosid® extended release 150-day briquets. No significant differences were observed between the control site and the methoprene treatment site in populations of water scavenger beetles[18] or predacious diving beetles (Hershey, 1995).

In a later, study Hershey *et.al.* studied the effects of *Bti* and methoprene applied for three consecutive years on non-target aquatic invertebrate communities of 27 wetland ecosystems in Wright County Minnesota. The authors state that in their sampling the following six **orders** were identified: **Diptera, Collembola, Bivalvia, Isopods, Annelida, and Gastropoda**. Of the 179 genera of aquatic species collected, 101 were from the **order Diptera** and 57 species of the Diptera were midges from the **family Chironomidae**. Hershey *et. al.* state that the reduction in predators on methoprene-treated sites, including both dipteran and non-dipteran may have been due to a combination of direct toxicity and changes in the food web. Community diversity was altered, whereby there was an overall reduction in species richness and an increase in the dominance of select species. Hershey concludes that observing these negative impacts on the invertebrate community, such as food web effects, requires that researchers reconsider the results of short-term tests showing no such adverse impacts (Hershey, 1998). While the authors maintain that the application was apparently consistent with the pesticide label, no information was provided on the methoprene concentrations found in the treated water.

(c) Crustaceans

Much concern has been raised regarding the potential for methoprene's use in larviciding activities to have an impact on crustaceans, such as shrimp, crabs and lobsters. These concerns are due to their shared evolutionary past and the resultant similarities in biology, as exemplified by the aquatic developmental cycles of insects in the order Diptera (mosquitoes and midges) to that of their distant relatives in the order Crustacea. These concerns have been further fueled by high profile incidents such as the 1999 Long Island Sound lobster die-off.

Most of the studies reviewed which looked at shrimp, Atlantic oysters, amphipods, copepods and mud crabs appear to indicate that adverse effects are not likely at or near the 10 ppb expected environmental concentrations. However the work done by EPA biologist Charles McKenney on grass shrimp and mysid shrimp suggest that there may indeed be reason for concerns at levels as low as 8 ppb and 2ppb.

Lobsters

Common in the media are reports that there might be an association between the 1999 lobster die-off in western Long Island Sound and the aerial application of the mosquito adulticide malathion or the use of methoprene in catch basins. According to the New York Times, however,

[16] The **order Odonata** includes damselflies and dragonflies, whose aquatic immature stage of development (naiads), are predaceous on mosquito larvae.

[17] Ostracods are crustaceans that have their entire body enclosed in the carapace, and resemble tiny clams.

[18] The **order Coleoptera** includes aquatic predators knows as water scavenger beetles from the **family Hydrophilidae.**

researchers have been skeptical of the pesticide theory, noting that while the lobster die-off peaked in the fall of 1999, it began in 1998, the summer before pesticide spraying began (October 29, 2000). During the same time period, there were unexplained mortalities of blue crabs and spider crabs in the sound and a continuing loss of sea urchins ranging from Massachusetts to Nova Scotia. This event also fell on the heels of another major lobster mortality event that occurred in Maine in 1997-98.

The New York Times also reports that 1,100 lobsterman from Long Island Sound have filed a lawsuit against five pesticide manufacturers, seeking $125 million in damages. The cause or causes of the "1999 Long Island Sound lobster die-off" are presently not well understood. Some scientists suspect that multiple factors may have played a role. The following is list of the possible factors, which may have played a role in the declining health of lobsters:

- Pollution stirred up from dredging materials such as PCB's, heavy metals and nitrates
- Unusually large outbreak of a deadly marine-born parasite[19]
- Shell disease[20]
- Aerial and ground spraying of pesticides for mosquito control[21]
- Low dissolved oxygen levels
- Excessive harvesting of lobsters in past years[22]
- Increased water temperature

According to the Sea Grant's Long Island Sound Lobster Initiative, University of Connecticut scientist Dr. Richard French and his colleagues have received grants totaling over $108,000 to perform a comprehensive study of the health of the lobsters in the sound. According to a March 31, 2001 New York Times article, University of Connecticut biologist Dr. Hans Laufer and seven colleagues will receive nearly $250,000 to conduct research into the possible link between the Long Island Sound lobster deaths and pesticides used to kill mosquitoes in the fight against West Nile Virus.

Mud Crab

Celestial and McKenney, 1994 studied the effects of methoprene on larval development of the mud crab (*Rhithropanopeus harrisii*). Larval development consists of four distinct zoeal stages and one megalopa stage prior to the first crab stage. The many similarities between this organism's aquatic development stage and that of mosquitoes make it a suitable non-target estuarine crustacean for such studies.

Larvae were treated with 0.1, 1.0, 10.0, 100.0, and 1,000.0 µg/L (ppb) methoprene. At methoprene concentrations of 1,000 µg/L (ppb) no larvae survived to megalopae. Significant reductions were found in survival at 100 µg/L (ppb) from hatch to megalops stage and from megalops stage to first crab stage. At concentrations less than 100 µg/L (ppb), no significant

[19] Dr. Richard French, a University of Connecticut pathologist found a one celled protozoan parasite, known as a paramoeba, which known to kill crabs and sea urchins, attacking the lobster's nervous system, causing limp lobster syndrome.

[20] Shell disease is a generic name for a variety of lesions found on shells of crustaceans.

[21] Ground and/or aerial adulticide applications related to WNV were made only in 1999 using malathion, sumithrin, and/or resmethrin.

[22] CT and NY officials estimated that there were more than 500,000 lobster pots in LIS in 1999. According to the National Marine Fisheries Service (NMFS), the lobster industry in New York hit a peak in 1996, with 9.4 million pounds caught, worth nearly $33 million, and stayed strong through 1998 , with 8.5 million pounds worth $29.8 million.

reductions were found in survival through developmental stages nor in survival among the same developmental stages across exposure concentrations.

In a study performed at the Duke University Marine Laboratory mud crab larvae were exposed to a maximum of 1,000 ppb methoprene. At optimal salinities for the mud crab (*Rhithropanopeus harrisii*) no effects were demonstrated in terms of molt frequency, duration of molt and molting time at concentrations $\leq$100 ppb. Waters with extreme salinities for the mud crab caused significant stress to the crab and thus increased its sensitivity (Costlow, 1977). This finding is later expanded upon in a later publication by Costlow where he found that reduced salinities caused 100% mortality in zoeal stages of the mud crab (*Rhithropanopeus harrisii*); however, when mud crabs were exposed to methoprene at 1,000 ppb but at salinities of 20 and 35 parts per thousand (ptt) survival was unaffected (Costlow, 1979). Based upon these findings it appears that salinity levels used in crab toxicity studies may play a critical role in the sensitivity of the test crustacean to various concentrations of methoprene.

Seawater salinity levels are typically 35.5 ppt fluctuate by about 4 %. Estuarine organism tend to be more tolerant of varying salinities; however it is possible that concentrations below 20 ppt would be too extreme for crabs and inappropriate for the study the methoprene toxicity in conditions typical of the crab habitat.

According the Massachusetts Division of Marine Fisheries tolerance to varying salinity concentrations in seawater is dependent upon a number of factors such as species, gender, season and temperature. Male blue crabs for example will move further into the mouth of the estuary than females where the salinity concentration will be lower. Female blue crabs will however, move further out towards and beyond the terminal part of the estuary. The recommended salinity range for maintaining American lobsters is 29-35 ppt (Bruce Estrella, Personal Communication).

In yet another study the mud crab (*Rhithropanopeus harrisii)* susceptibility to methoprene was again studied in the laboratory using 10, 100, and 1,000 ppb of methoprene with various salinity 5-35 ppt and temperature (20-35^0 C), these authors found a significant reduction in the survival of zoeal larvae with increasing methoprene concentrations at almost all temperature/salinity combinations. One thousand parts per billion (1,000 ppb) completely arrested further development. At under 100 ppb little effect on metamorphosis was noted (Glare, 1999).

Blue Crab

Horst and Walker, 1999 studied the effects of methoprene on morphogenesis and shell formation of the blue crab (*Callinectes sapidus*). In this study samples of post-molt female crabs were used to isolate exoskeleton labeling to study protein and chitin synthesis; stage 2-4 embryos were used to study uptake of methoprene and development in the presence of methoprene; and megalopae (post larvae) were utilized for molting characteristics. The four components of this study and their findings are as follows:

- *Electron Microscopy Study of Internal Cellular Organellar Organization*
  Upon examination via electron microscopy, treatment at 1,500 ppb methoprene caused profound ultrastructural changes in the cuticular epithelial cells (exoskeleton) of postmolt adult blue crabs studied in vitro; these changes included loss of secretory organelles as well as swelling (distention) and air sacs (blebs) of the outer membrane of the nuclear envelope.

- *Radiolabel Explant Tissue to Study Protein and Chitin Synthesis*
  For protein and chitin synthesis, explant post-molt carapace cultures were treated with 1,500 ppb methoprene for 8–22 hours including a 2 hour label with either [^{35}S]-methionine or [^{3}H]-glucosamine. The authors demonstrate an approximate 50% reduced synthesis of radiolabeled proteins in cuticle new growth and a contrasting >400% increased synthesis of radiolabeled proteins in the epithelial cells.

- *Hatchability and Survivability of Embryos and Methoprene Uptake*
  Stage 2-4 embryos were treated with 300 – 1,500 ppb methoprene under static conditions for up to 11 days prior to performing morbidity/mortality and hatchability analysis. Treatment with 5750 ppb, 1,500 ppb or 300 ppb methoprene resulted in 61%, 25%, or 54% hatching rate respectively compared to approximately 75% in controls.

- *Megalopae Morbidity and Mortality*
  Megalopae (post-larvae) were continuously exposed to 500 µg/L (ppb). After 10 days, 80% mortality in methoprene treated megalopae is observed compared to 25% mortality in control animals.

The authors state that at concentrations likely to be seen in the environment, methoprene produced morbidity and mortality. This statement however is inaccurate and misleading. Exposures of 300 to 1,500 ppb methoprene are far greater than typical field use rates which do not exceed expected environmental concentrations of 10 ppb (Ross, 1994). In addition, without additional supportive data, the *in-vitro* (outside the whole living organism) part of this study examining impacts on protein synthesis is not indicative of findings from whole animal toxicity studies.

Earlier laboratory research indicates that at salinities of 20 and 35 parts per thousand (ptt), 100% mortality of megalopa stage of blue crab (*Callinectes sapidus*) was observed when animals were exposed to 10,000 µg/L (ppb). In the same range of salinities survival was reduced to 40% when animals were exposed to 1,000 µg/L (ppb) (Costlow, 1979). As mentioned earlier the expected environmental concentrations of methoprene from labeled applications is 10 µg/L (ppb). Thus the toxicity of methoprene to the blue crab at concentrations of 10,000 µg/L (ppb) and 1,000 µg/L (ppb) are not indicative of potential effects at expected environmental concentrations.

Shrimp

A laboratory study was completed for Zoecon Corporation examining the acute toxicity of Altosid® technical (90.7%) to the estuarine grass shrimp *(Palaemonetes pugio).* Altosid® was not found to be acutely toxic to grass shrimp at nominal concentrations up to 10 ppm (Bionomics EG&G, Inc. 1975).

In a 1992 the acute toxicity of (S)-methoprene to the mysid shrimp (*Mysidopsis bahia*) was studied for Zoecon Corporation by Springborn Laboratories, Inc. Wareham, Massachusetts. Twenty mysid shrimp per treatment group were exposed to mean measured concentrations of technical (S)-methoprene at 150, 84, 35, 17, and 10 µg/L (ppb) under flow-through conditions. At test termination (96-hours), a mortality rate of 85 and 25% was observed among organisms exposed to the 150 and 84 µg/L (ppb) mean measured test concentrations, respectively. Sublethal effects (e.g., lethargy, losss of equilibrium) were also observed among several of the surviving mysids exposed to these two test concentrations. No mortality or sublethal effects were observed among mysids exposed to the remaining concentrations tested [35 - 10 µg/L (ppb)]. The calculated LC_{50} values are >150, >150, and 110 µg/L (ppb) for 24-hours, 48-hours,

72-hours and 96-hours respectively. The No Observed Effect Concentration (NOEC) established for this study was determined to be 35 µg/L (ppb). The risk quotient (RQ) value calculated in **Table Four** (below) indicates minimal risk of acute toxicity to shrimp (Machado, 1992).

Table Four: Risk Quotients for Mysid Shrimp (*Mysidopsis bahia*) and tadpole shrimp *(Triops longicuadatus)* (Machado, 1992 and Miura, 1973).

Animal	State of growth	Number of tests	Number Animals per container	Test Duration (hours)	LC_{50} (ppm)	Acute RQ[23] (EEC/LC_{50})[24]
mysid shrimp (*Mysidopsis bahia*)	juvenile	2	20	24	>150	6.6×10^{-5}
Tadpole shrimp *(Triops longicuadatus)*	1.2 cm immature	8	10	24-96	5.00	0.002
clam shrimps *(Eulimnadia sp.)*	Mixed	3	10	24	1.00	0.01

In 1996 chronic toxicity of (S)-methoprene to the mysid shrimp (*Mysidopsis bahia*) was studied for Zoecon Corporation by Springborn Laboratories, Inc. Wareham, Massachusetts. The Lowest Observed Effect Concentration (LOEC) established for this study was determined to be 25 µg/L (ppb).The No Observed Effect Concentration (NOEC) established for this study was determined to be 14 µg/L (ppb). The Maximum Acceptable-Toxicant Concentration (MATC)[25] was calculated to be >14 µg/L (ppb) and <25 µg/L (ppb) or the mean of 19 µg/L (ppb) methoprene (Sousa, 1996).

McKenney and Mathews from the U.S. EPA studied the larval development of the estuarine grass shrimp *(Palaemonetes pugio)* exposed to methoprene. In this laboratory study larvae were reared in nominal concentrations of 0.1, 1, 10, 100, and 1,000 µg/L (ppb) of either isomer, (R,S)-methoprene or (S)-methoprene. No grass shrimp larvae survived completion of metamorphosis with exposure to 1,000 µg/L (ppb) regardless of the isomer used. Larval survival was significantly reduced by exposure to 100 µg/L (ppb) (RS)-methoprene but not by this concentration of (S)-methoprene. No significant difference was revealed, however, in ability to inhibit metamorphosis between these two isomeric types across the broad range of exposure concentrations from 0.1 to 1,000 µg/L (ppb). Methoprene exposure did not alter either the duration of total larval development or the total number of larval stages prior to metamorphosis *(McKenney, 1990)*.

[23] Risk Quotient (RQ): The estimated environmental concentration / median lethal dose (LC_{50}); The lower the Risk Quotient (RQ) the less risk.
[24] According to Ross *et.al.* 10 µg/L (ppb) or 0.01 mg/L (ppm) is the Expected environmental concentrations from the application of ALTOSID Liquid Larvicide at 4 fluid oz. /acre (293 ml/ha) (Ross, 1994).
[25] Maximum Acceptable Toxicant Concentration (MATC) is the hypothetical toxic threshold concentration lying in a range bounded at the lower by the highest tested concentration having no observed effect (NOEC) and at the high end by the lowest concentration having a significant toxic effect (LOEC) in a life cycle (full chronic) or partial life cycle (partial chronic) test. This may be represented as NOEC < MATC < LOEC. Calculation of MATC requires quantitative life cycle toxicity data on the effects of a material on survival, growth, and reproduction.

McKenney and Celestial from the U.S. EPA studied the growth and metabolism of the estuarine grass shrimp *(Palaemonetes pugio)* exposed to methoprene. According to the researchers, exposure to methoprene at concentrations ≥8 µg/L (ppb) through larval development inhibited successful completion of metamorphosis. Methoprene retarded growth in early larval stages and post-larvae, but enhanced growth in pre-metamorphic larvae. Respiration rates of early larvae were elevated by methoprene, but not so in older larvae or post-larvae. Lower net growth efficiency in methoprene-exposed early larvae suggests that increased metabolic demands reduced assimilated energy available for growth. Responses of developing grass shrimp larvae to methoprene are characteristic of those of insects to juvenile hormone (McKenney, 1992).

In other laboratory studies, McKenney and Celestial studied the survival, growth and reproduction of estuarine mysid shrimp *(Mysidopsis bahia*[26]*)* exposed to (S)-methoprene through a whole life cycle. According to the authors, total lethality occurred among all juvenile mysids exposed to 125 µg/L (ppb) for 4 days. Mysids reared at the sublethal concentration of 62 µg/L (ppb) weighed significantly less than unexposed mysids as they matured after 15 days of exposure. Release of the first brood was significantly delayed by as much as 3 days in mysids exposed to low µg/L (ppb) concentrations. The total number of young produced by groups of mysids during their first brood was significantly reduced when mysids were reared in methoprene concentrations ≥8 µg/L (ppb). The most sensitive response of mysids to methoprene exposure was a significant reduction in the number of young produced per female in concentrations ≥2 µg/L (ppb) *(McKenney, 1996)*.

Water Fleas

In a study examining the acute and chronic effect of (S)-methoprene (Altosid®) on water fleas[27] (*Moina macrocopa*) the 24- and 48-hour LC_{50} values were calculated to be 510 and 340 µg/L (ppb) respectively. Water flea survival, longevity, and fecundity were reduced at 50 µg/L (ppb) and higher concentrations. At 5 and 10 µg/L (ppb) longevity, and fecundity increased slightly as compared to controls. The authors state that if environmental concentrations do not exceed 50 µg/L (ppb), which is likely the case, application of this insecticide is unlikely to cause detrimental effects on natural water flea populations. They conclude that the reproductive stimulation of methoprene on the water flea, as observed at 5 and 10 µg/L (ppb), is consistent with the following hypothesis: that juvenile hormone analogs, such as methoprene may affect reproduction and development in crustaceans because methyl farnesoate, a juvenile hormone endogenous to crustaceans, is believed to play a regulatory role in these processes and has a chemical structure similar to that of the insect juvenile hormone (Chu, 1997).

In another study, the short-term effects of ZR-515 [(RS)-methoprene] were tested on the *Daphnia magna* and *Cyclops sp.* in the laboratory and in outdoor artificial containers. *Daphnia magna* and *Cyclops sp.* were maintained in aquariums treated with 10% Flowable liquid formulation (slow release) of ZR-515 and contained 0.1 ppm (RS)-methoprene. According to the authors *Daphnia sp.* showed the least tolerance to technical ZR-515 in the laboratory tests with 24-hr LC_{50} value of 0.90 ppm. The calculated risk quotient values in **Table Five** (below) indicate minimal risks for acute toxicity to water fleas (Miura, 1973).

[26] *Mysidopsis bahia* has been shown to be one of the most sensitive members of the estuarine community to a variety of pesticides.

[27] Water fleas are crustaceans from the **order Cladocera** that are sometimes extremely abundant in freshwater pools. *Moina* appear in high concentrations in pools, ponds, lakes, ditches, slow-moving streams, and swamps where organic material is decomposing and are ideally suited for feeding freshwater fish fry.

Copepods
Copepods are one of the most abundant animals on the planet. Most are saltwater plankton, living their entire lives in the open ocean without ever touching the bottom or surface. Copepods also live on the sea bottom, in fresh water, as parasites on fish, or in caves. Copepods are important components of the food chain in aquatic systems and some species are predatory on mosquito larvae. In the previously discussed Miura and Takahashi 1973 study, the authors calculated an LC_{50} value 4.60 mg/L (ppm) for copepods *(Cyclops sp.)*. The calculated risk quotient values in **Table Five** (below) indicate minimal risks for acute toxicity to copepods (Miura, 1973). Subsequent studies by Schaefer *et al.* also found no effect of methoprene (Altosid®) on water fleas *(Daphnia* and *Moina* spp.) and a variety of copepods (Glare, 1999).

Table Five: Risk Quotients for Water Fleas *(Daphnia magna sp.)* and Copepods *(Cyclops sp.)* (Miura, 1973).

Animal	State of growth	Number of tests	Number Animals per container	Test Duration (hours)	LC_{50} (ppm)	Acute RQ (EEC/LC_{50})
Water Fleas *(Daphnia magna sp.)*	Mixed	3	30+	24	0.90	0.011
Copepods *(Cyclops sp.)*	Mixed	3	30+	24	4.60	0.002

Amphipods
Amphipods are considered an important food constituent of fish and some share their breeding places with mosquitoes. In an acute toxicity study with the amphipod (*Gammarus Aequicauda)* serial dilutions of technical Altosid® (65.8%) were prepared. Ten animals per concentration were utilized and LC_{50} and LC_{90} values were calculated **(Table Six)**.

Table Six: Risk Quotients for Amphipods (*Gammarus Aequicauda)* (Gradoni, 1976).

Stage	LC_{50} (ppm)	LC_{90} (ppm)	Test Duration (hours)	Acute RQ (EEC/LC_{50})[28]
Adult females	2.15	4.10	96	0.004
Adult males	1.95	7.80	96	0.005
Young	0.32	1.05	24	0.031

Based on the LC_{50} values above, it appears that the young amphipods are 6-7 times more sensitive to methoprene than adult amphipods of the same species; however calculated risk quotient values indicate minimal concerns for acute toxicity (Gradoni, 1976).

(d) Atlantic Oyster (Phylum, Mollusca)
In a 1972 the acute toxicity of (R,S)-methoprene (technical ZR-515, 69.7% active ingredient) to the Atlantic oysters *(Crassostrea virginica)* was studied for Zoecon Corporation by Bionomics

[28] According to Ross *et.al.* 10 µg/L (ppb) or 0.01 mg/L (ppm) is the Expected environmental concentrations from the application of ALTOSID® Liquid Larvicide at 4 fluid oz. /acre (293 ml/ha) (Ross, 1994).

Inc., later known as Springborn Laboratories, Inc. The goal of the study was to determine a 48-hour median tolerance limit (48-hr TL_{50}), which is defined as the concentration of the chemical in water which causes 50% response under the test conditions during a 48-hour interval.

The response observed in these studies was normal embryonic development. For observations on development of embryos, fertilized eggs were introduced into the test container soon after release. Quantitative samples were taken 48 hours later to determine the percentage of the fertilized eggs that had developed to a normal morphological stage (i.e. straight-hinged veliger larvae).

The predicted 48-hour TL_{50} (i.e. concentration of Altosid® which inhibited normal development 50% of the developing oyster larvae) was 0.247 mg/L (ppm). No effect on normal embryonic development was observed among oyster larvae exposed to Altosid® at 0.075 mg/L (ppm) for 48-hours (Sleight, 1972).

(e) Fish

According to the June 2001 Update of the 1991 EPA Registration Eligibility Document, methoprene poses minimal chronic and acute risks to freshwater fish. Table Seven provides a summary of toxicity endpoints to fish.

The EPA 1991 R.E.D. summarized available fish studies concluding that methoprene is moderately toxic to warm water, freshwater fish and slightly toxic to coldwater, freshwater fish. Exposure of fish to methoprene has produced LC_{50} values ranging from 3.3 mg/L (ppm) for trout to >100 mg/L (ppm) for channel catfish *(Ictalurus punctatus*). Acute fish toxicity would not be expected during control programs as the concentration of methoprene in water at any one time is unlikely to exceed 10 µg/L (ppb) (Ross 1994). It should also be noted that some of the experimental work examining methoprene toxicity to fish used special solvents, such as dimethyl-formamide, to increase methoprene's solubility in water. Solvents are not used in Altosid® formulations and the solubility of methoprene is approximately 1.39 mg/L (ppm) (Glare, 1999).

Table Seven: Toxicity of Methoprene to Various Aquatic Organisms (Hicks, 2001).

AI	WARM WATER FISH LC_{50} (MEDIAN LETHAL CONCENTRATION)	COLD WATER FISH LC_{50}	ESTUARINE AND MARINE TOXICITY	FRESHWATER INVERTEBRATES
Methoprene [3]	Bluegill sunfish: 96hr LC_{50} 1,520ppb [3] 96 hr TL_{50} (median threshold limit) = 4,600 ppb (static) [2] LC_{50} > 370 ppb [3] Channel catfish: TL_{50} > 100,000 ppb (static) [54] Fathead minnow: LEL (Lowest Effective Level) = 84 ppb [22b] NOEL = 48 ppb [22b]	Rainbow trout: 96 hr LC_{50} > 50,000 ppb[3] Juvenile Rainbow trout: LC_{50} = 106,000 ppb [54] LC_{50} = 760 ppb [22b] LC_{50} = 106,000 [54] Trout: TL_{50} = 4,400 ppb (static) [54] TL_{50}= 106,000 ppb (static aerated) [54] Coho salmon LC 50 = 86,000 ppb [54]	Mud crab: ↓ gametes in @ 1,300 ppb [3] Adult grass shrimp: Slightly toxic [3] not acutely toxic [41] Juvenile grass shrimp and larval mud-crabs: Very highly toxic [1] not acutely toxic Gammarus aequicauda: 96 hr LC_{50} = 2,150 ppb (females) [54, 22d] 96 hr LC_{50} = 1,950 ppb (males) [54, 22d] Mysid Shrimp: 96 hr LC_{50} = 110 ppb [22b] 28 day MATC = > 98 ppb [22b] Oyster (larvae): 48 hr LC_{50} = 247 ppb [22b] Oyster shell deposition 96 hr = 1,400 ppb [22b]	Daphnia; 48 hr EC_{50} 89 ppb [3] 42 day MATC 27 - 51 ppb [3] 48 hr EC_{50} = 360 ppb [22b] 42 day MATC 51 ppb [22b]

[3] EPA (1991) R.E.D. Methoprene.
[22b] Sandoz (1996) Submission of Environmental Toxicity and Release Data to EPA.
[22d] Grandoni, L., Bettini, S. and Majors, G. 1976. *Toxicity of Altosid® to the Crustacean: Gammarus aequicauda*. Mosquito News, Vol. 36(3):294-297.
[41] Wellmark (2001) Comments on March 5,2001 Maine draft report by Hicks, Lebelle:
[54] Vershcueren, K. Handbook of Environmental Data on Organic Chemicals. 2nd Ed. Van Nostrand Reinhold Press, NY, 1983.

4. ENVIRONMENTAL FATE AND TRANSPORT

Methoprene is rapidly degraded under field conditions. The biological activity of aqueous solutions of methoprene has been shown to be affected by sunlight, temperature, and microbial action. The metabolism of methoprene by aquatic microorganisms is extensive, with methoxycitronellic acid being the major microbial breakdown product. The most abundant photoproduct in aqueous solution is methoxycitronellal (9%). While methoprene degrades rapidly in the field, the persistence in water is determined, ultimately however, by the formulation and method of application. The XR briquet releases small amounts of methoprene as it degrades over a period of 150 days. While the briquet has been shown to physically persist in water for eighteen months, no data is available to indicate biological activity for this time period.

Stability in water

Methoprene is extremely stable to hydrolysis (Schooley, 1975). The principal modes of degradation in water are photodegradation and degradation by aquatic microorganisms (EXTOXNET, 1996). Quistad has shown in the laboratory that the photolysis half life of methoprene in water is less than one day (Quistad, 1975). In field trials, aqueous solutions of methoprene formulated as an emulsifiable concentrate were found to have a half life of only about two hours (Schaeffer, 1973). Treatment of a ten percent flowable liquid (slow release) formulation in water showed almost no detectable residues after 24 hours, although biological activity persisted for several days (Schaeffer, 1973). Studies under conditions that approximate the natural environment and the use rates of methoprene for mosquito control, have demonstrated half lives in pond water of about 30 and 40 hours at initial concentrations of 0.001 ppm and 0.01 ppm respectively (Schooley, 1975). Measuring the methoprene concentration in the field is difficult due to its rapid rate of dissipation.

Because of its rapid rate of degradation, several formulations of methoprene have been developed which extend the active life of methoprene in water. As shown in Table Two, these formulations include slow release solids such as briquets, pellets and granules. While these slow release formulations provide excellent control of mosquito larvae, they have been shown to physically degrade over a period of as long as eighteen months (Boxmeyer, 1997).

Methoprene, formulated as slow release Altosid® briquets in a Minnesota study was found under field conditions to degrade on average to 19% of its weight within 150 days of immersion in water. The briquet however, took eighteen months to completely degrade. No information in the study was provided to indicate whether the briquet continued to be biologically activity for eighteen months (Boxmeyer, 1997).

However, Ross *et al* of Zoecon Corporation maintain that methoprene has a short environmental persistence when applied in sustained release formulations. In an experiment to measure the methoprene concentrations present over time in aquatic microcosms treated with the liquid larvicide and with sustained release Altosid® formulations – Altosid® Briquets (AB), XR Briquets, and Pellets-, no sample collected in the experiment contained a residue in excess of 10 µg/L (ppb)[29]. In fact, 85% of the residues in the 186 samples analyzed were less than 1 µg/L (ppb). Water samples were taken at various intervals over a 35 day period. The study found that the highest methoprene concentrations from the use of Altosid® liquid larvicide, 2.2 µg/L(ppb), is produced at days 1 and 3 post application. The 30 day briquets (AB) and the 30 day pellets

[29] The expected environmental concentrations of methoprene produced by the application of ALTOSID® Liquid Larvacide at the maximum rate of 4 floz/0.5 acre feet of water is 10 µg/L (ppb).

produce peak methoprene concentrations of 4 µg/L(ppb) and 2µg/L(ppb) at seven days. For the 150 day briquets (XR), the concentration remained consistently around the 0.2 µg/L(ppb) level for the 35 day study peaking at 0.7 µg/L(ppb) at day two (Ross *et al*, 1994).

In its 1991 RED, EPA expressed concern over the use of the slow release formulation because it causes estuarine organisms to be exposed to methoprene over an extended period of time (EPA, 1991). And in fact, New York state has limited the use of the 150 day briquet based upon similar concerns. Studies submitted by Wellmark International subsequent to the 1991 RED have led to the removal of any such language from the federal label. However New York continues to prohibit the use of the slow release formulation under specific circumstances. This issue is discussed in more detail in the following section which addresses the varying approaches taken at the state and federal level to regulate methoprene use.

Aquatic Microorganisms

Schooley *et al* report that the metabolism of methoprene by aquatic microorganisms is extensive and appears to occur more rapidly than the competing photo-initiated decomposition of methoprene in solution (Schooley, 1975). The major microbial product is 7-methoxy-3, 7 dimethyloctanoic acid (methoxycitronellic acid). However the contribution to degradation of each process under field conditions is likely to be determined by the specific environmental conditions.

Light

Methoprene degrades rapidly in sunlight, both in water and on inert surfaces (Scheaffer, 1973, EPA, 1991). It undergoes photolytic degradation to a large number of photoproducts all of which are present in relatively low yield (< 10 %) (Quistad, 1975). After one week of irradiation, an aqueous solution of methoprene yielded four major photoproducts. The most abundant photoproduct was 7-methoxycitronellal (9%). The other products included methoxycitronellic acid (7%), an epoxide of methoprene (4%), and a methyl ketone (4%). At least 46 other products were detected, with none representing more than a 2% yield. Unreacted methoprene was not detected after two weeks of exposure to sunshine (Quistad, 1975).

Methoprene is also susceptible to photoisomerization of the 2-ene double bond to various mixtures of photoisomers. Schaeefer has reported that sunlight reduces the biological activity of methoprene (Schaefer, 1973). This is due to the fact that the *2E, 4E* isomer is readily converted to the biologically less active *2Z,4E* isomer in solution (Quistad, 1975). The *2Z,4E* isomer is approximately 1000 times less active on mosquito larvae. Interestingly, a 1:1 isomeric ratio of *2Z,4E* to *2E,4E* was consistently observed after field exposure of methoprene to sunlight. Consequently, a non-degradative loss of about half the biological activity of methoprene should result from photoisomerization. With slow release formulations, however, biological potency is maintained over longer periods.

Temperature

Schaefer has determined that temperature has a definite effect. At 12°C, a solution of methoprene averaged 21% of the initial concentration after 8 hours. For a solution where the water temperature reached 39°C , the concentration was reduced to 1.3% after the same exposure. While high temperatures do have an effect, Schaefer concluded that sunlight appears to be a much more important factor accounting for a rapid decline in concentration in laboratory experiments (Schaeffer, 1973).

6. GOVERNMENT REGULATION

USEPA has registered all commercially available Altosid® formulations of methoprene for use in fish bearing waters. However, the 1991 EPA RED factsheet states that methoprene is highly acutely toxic to estuarine invertebrates. EPA has recently updated this R.E.D. (June 2001), concluding that:

- **all ecological concerns contained in the 1991 R.E.D. factsheet related to toxicity to estuarine invertebrates have been alleviated as a result of the submission of studies which indicate a minimal chronic risk to Mysid Shrimp, and**
- **all methoprene end–use products have completed the registration process.**

New York prohibits the application of sustained-release formulations to fish bearing waters. Maryland imposes conditions on the application of methoprene to water bodies on a case by case basis. While Altosid® is registered for use in fish bearing waters in all other states, a number of states have placed limitations on the application of methoprene products, along with dozens of other pesticides, to waters containing endangered species.

Federal Government

Conflicting messages from EPA regarding the use of methoprene in aquatic environments have confused the issue of methoprene use in fish bearing waters. The 1991 EPA Registration Eligibility Decision (R.E.D.) factsheet stated that "the ecological effects studies on methoprene suggest that use of the briquet or slow-release formulation in estuarine areas may cause undue risks to estuarine invertebrates, since the pesticide is highly toxic to these organisms." However the label contains no such language and methoprene is routinely used for insect control in aquatic environments. The reasons for this discrepancy arise out of the complexities inherent to the federal pesticide product registration process. However in June 2001, EPA issued an updated R.E.D. factsheet which concludes that:

- the studies available to EPA indicate that the biochemical insect growth regulator Methoprene is of low toxicity and poses very little hazard to people and other non-target species,
- ecological concerns contained in the 1991 Methoprene R.E.D. factsheet related to toxicity to estuarine invertebrates have been alleviated as a result of the submission of the estuarine invertebrate life cycle toxicity study in 1996, which indicated minimal chronic risk to Mysid Shrimp,
- all Methoprene end-use products completed the reregistration process in 1997 and all reregistration data requirements and label changes have been completed.

The issue is further complicated because New York state continues to require that the slow release formulations bear the label with the statement prohibiting applications to fish bearing waters. Maryland imposes conditions on methoprene use, specific to the resource, through a pesticide aquatic application process. Several other states have placed limitations on the application of methoprene (along with dozens of other commonly used pesticides) to waters containing endangered species. The registration status of methoprene in several states, including New York, is further discussed in the following section.

New York

New York is the only state that currently requires Wellmark International to distribute and sell their slow-release formulations with amended labels, unique to New York, which prohibit any applications to fish-bearing waters. Based upon a recent risk assessment of the slow release

formulations conducted by the Department of Environmental Conservation (DEC), a decision has been made to maintain this prohibition.

According to Tim Sinnott, Ecotoxicology and Standard Unit Leader (Personal communication, July, 2001), DEC has determined that methoprene itself is not acutely toxic to fish and amphibian species tested at concentrations likely to be encountered in the environment following a labeled application. However, the DEC risk assessment expressed concerns that certain methoprene degradates could potentially be teratogenic to amphibians. In addition, the Boxmeyer study (described on page 15) which reported that Altosid® briquets degraded over an eighteen month time period fueled further concerns among environmental risk managers in New York regarding the persistence of methoprene's degradation products. These concerns centered on the teratogenic compounds that could potentially be continuously generated as a result of the sustained release of methoprene over time into a water body. Because the briquet degrades over such a long period, DEC also expressed concerns that the briquet could over winter potentially becoming active again in the early spring at a time when amphibians and fish typically spawn. These findings provided the DEC with enough concerns about the long term effects to recommend that the amended label for the slow release formulations should remain unchanged. While they have no definitive evidence that methoprene degradates are teratogenic, the outcome of the risk assessment was such that they felt that they could not rule out the possibility (of teratogenicity) based upon the data reviewed.

The use of methoprene, including sustained-release formulations, is an integral part of the New York's Routine Comprehensive Arthropod-borne Disease Surveillance and Control Program. According to this program, Altosid® briquets and Altosid® granules will be applied to non-seepage storm drains or catch basins. Non-seepage drains are basins that do not allow the water to drain into the ground. Seepage basins that allow the water to eventually seep into the ground will be treated with VectoLex (*Bacillus sphaericus*). This plan also states their intention to apply VectoLex (*Bacillus sphaericus*) and VectoBac (*Bti*) to freshwater ponds, lakes, and numerous other areas determined to be sensitive natural resources. This plan does allow the option of applying the Altosid® Liquid Larvicide formulation of methoprene to such sensitive natural areas on a site by site basis.

New York's Division of Fish, Wildlife and Marine Resources further recommends that the extended release briquet should not be used in waters inhabited by endangered species.

New Jersey

New Jersey has one of the most comprehensive mosquito control programs in the Northeast and reports that no restrictions beyond the label exist on the use of any Altosid® products. County projects, which use Altosid® Liquid Larvicide as their primary larvicide on salt marshes, report that it provides excellent control of mosquito emergence.

Maryland

Maryland does not have a direct prohibition on the use of sustained release formulations to fish bearing waters. Instead, Maryland imposes specific conditions and restrictions on methoprene use, on a case by case basis, through the Toxic Materials Permitting process of the Department of the Environment. These conditions are based upon a recommendation to the Department of the Environment (DOE) from the Department of Natural Resources (DNR). MDE has reviewed data that show methoprene can impact certain non-target organisms, but they recognize that the concentration and duration of exposure to cause these effects are far greater than will occur

following application of methoprene for mosquito control per label directions (Personal Communication, Cyrus R. Lesser, Chief Mosquito Control Section, MDA).

An example of typical conditions, drawn from Maryland Toxic Permits TMP-01-127 which was issued on March 2, 2001, includes the following points:

- There shall be no direct application of methoprene to Use III waters, headwater tributaries, or contiguous wetlands. Between March 1 and June 15, there shall be no direct application of methoprene to documented anadromous finfish spawning areas or their contiguous wetlands.

- Use of residual formulations of methoprene is restricted to storm water detention facilities and isolated woodland pools, neither of which is within or immediately next to estuarine aquatic habitat areas (including marshes).

- **Specific areas of Concern:** There shall be no direct application of methoprene to wetlands or conveyances directly contiguous to the tidal marsh along Deep Creek, just north of Franklin Manor.

Placing limitations on methoprene use in Maryland is a source of contention between the Maryland Department of Agriculture (DOA) and the Department of Natural Resources.

Maine

Altosid® products are registered for use in all New England states. However, whereas each state has a different arbovirus or mosquito control program, registration of a larvicide does not necessarily equate to use. Maine for example does not have an organized mosquito control program. Applications of any larvicide including *Bti, Bs,* and methoprene to a salt marsh require a pollution discharge permit from the Maine Department of Environmental Protection (DEP). Beyond the permitting process there are no additional restrictions on the registration of methoprene in Maine. Maine has restrictions on the application of any material to water that is not contained and enters into another body of water or may be used by the public. DEP does not normally give out any permits for application of pesticides to bodies of water that empty into a pond, lake, or river. Without such a permit only registered materials may be applied to water bodies that are contained on an individual's property and do not connect to other bodies of water.

New Hampshire

Although there are no additional restrictions on the registration of Altosid® products in the state of New Hampshire, a permit from the New Hampshire Department of Agriculture's, Division of Pesticide Control is required to apply a pesticide to or within 25 feet of any body of water, including vernal pools. Although permits are issued on a case by case basis, there is a limited number of municipalities such as Hampton, Hampton Beach, and Rye which have organized mosquito control programs that require permits for season long applications of larvicides such as Altosid® to saltwater marsh areas and catch basins. Several factors must be present however, prior to the issuance of such permits. For example, municipalities must work in cooperation with someone who is certified to use pesticides and is specially trained in pest identification and surveillance techniques.

Vermont

Any application of a pesticide to water requires a permit from the Vermont Division of Water Quality. In Vermont however, the Department of Agriculture provides permits for any larvicide/ mosquito related applications. Vermont has one organized mosquito control district involving

four towns. Recent studies in Lake Champlain have documented the occurrence of malformed *Rana pipiens*. As a result of these malformations methoprene applications will be restricted and may be made only on a case by case basis as approved by the Department of Agriculture. Permits will be granted for the application of Altosid® Briquets to catchbasins and vernal pools. According to John Turmal 99% of mosquito control applications use *Bti*. In 2000, EPA issued a request for applications under the Regional Applied Research Effort (RARE) program to conduct investigations into the possible causes of amphibian malformations in Lake Champlain.

Rhode Island

There are no additional restrictions on the use of methoprene in the state of Rhode Island. The program in Rhode Island provides oversight and training to communities and shares mosquito control activities, such as larviciding, with hired commercial applicators and certified individuals working for municipalities.

Connecticut

Beyond the label language, there are no additional restrictions on the use of methoprene in Connecticut. However, any application of a chemical to water for control of aquatic organisms in state waters, including catch basins, requires a permit. Biological pest control agents such as *Bti* and *Bs* are exempt from this permitting process. Connecticut's organized mosquito control program is held within its Department of Environmental Protection. The activities of pesticide related activities of the DEP are exempt from the permitting process. According to Roger Wolff of DEP, Altosid® products are used for salt marsh mosquito control along the coast of Connecticut (Personal Communication, July 2001).

Florida

Methoprene is widely used in the state of Florida where there are no additional restrictions on its use. The low toxicity of Altosid® to non-target organisms, makes it the preferred product for use in Florida's State and Federal Parks. However according to Dr. Jonathan Hornby of Applied Science and Technology, Abate (temephos) is currently the main larvicide used in many of Florida's salt marsh lands due to its relatively low cost. Altosid® however, is used extensively in pastures, retention ponds and other areas due to restrictions on the Abate label.

In work yet to be completed and published Florida officials observed increased emergence of their primary saltwater mosquito pest *(Ochlerotatus taeniorhynchus)* after using Altosid® XR Briquets over a four year period. As the location of this study site was off shore, the island conditions provided the opportunity to study the effectiveness of Altosid® on this species without the migration of outside species and their genes into the study population. The location provided an optimal natural setting for studying the potential development of resistance. Florida officials have not observed mosquito resistance from their use of temephos (Personal Communication, Bryan Smith, Larviciding Field Supervisor and Jonathan Hornby, Ph.D. Div. Head Applied Science and Technology, Lee County, Florida).

Other States

Several states such as, Alabama, Arkansas, Kentucky, Mississippi, Nebraska, Nevada, Oklahoma and Virginia have at the time of writing placed limitations on the application of methoprene to waters containing endangered species. However, all of these states have placed similar limitations on the use of dozens of pesticides – 63 active ingredients in the case of Hinds County, Mississippi- in the vicinity of waters containing endangered species. The limitations vary considerably from state to state. In Hinds County, Mississippi, the limitation on methoprene use amounts to a recommendation to read some general information about reducing runoff and drift.

In Lee County Virginia, methoprene cannot be applied within 20 yards of the water's edge or within 100 yards for aerial applications. Similar restrictions apply to benomyl, captan, carbaryl, chlorpyrifos, diazinon and a dozen additional pesticides.

8. CONCLUSIONS

Methoprene is one of the most effective tools available for midge and mosquito control. It is used in Massachusetts, most notably, to control mosquito larvae in catch basins as part of municipal West Nile Virus prevention strategies. However it has come under considerable scrutiny over the past few years due to its suspected role as a causative agent in amphibian deformities. It has been further implicated in a die off of lobsters in Long Island Sound in 1999. Despite the fact that it has been registered for use since 1996 on all water bodies, doubts persist about its long term effects among the scientific community and some state regulators. Our findings can be summarized as follows:

- We have found no evidence to suggest that the labeled application of methoprene for mosquito and midge control will lead to amphibian malformations. Methoprene and its breakdown products are not retinoid compounds. Methoprene *has* been shown in the laboratory to break down into methoprenic acid which can mimic retinoid activity, and cause deformities in frogs at extraordinarily high levels. However, methoprenic acid is not found in the natural environment. There is far greater body of evidence that suggests that parasitic trematodes and ultra violet light may be more likely causative agents.

- Studies reviewed observed variable susceptibilities of crustaceans to methoprene. Several short term studies indicated no effect from methoprene on aquatic invertebrate crustaceans such as the Blue Crab, the Mud Crab, Shrimp and some varieties of Copepods. However, short term studies on the fresh water crustacean Daphnia Magna provide cause for concern. A longer term chronic study fuels concerns for the potential toxicity of methoprene, at levels which might be expected from a labeled application, to Grass Shrimp Larvae. At this time, it is difficult to draw final conclusions regarding the safety of methoprene for crustaceans until further research is completed and available for review. The weight of evidence reviewed, however, suggests that impacts upon crustraceans are not likely at expected environmental concentrations.

- Because the half life of methoprene is quite short, the use of the liquid larvicide is unlikely to create any adverse impacts. Possible exceptions are repeated applications, or the use of methoprene slow release formulations in shallow, poorly flushed waters. Water sampling show that minute concentrations (~0.2 ppb) of methoprene result from the use of this briquet over a 35 day period. The highest concentration (0.7 ppb) is found at day two. However, the briquet is formulated to remain active for at least 150 days. It has been shown to be physically present for up to 18 months. It is unlikely to present any adverse effects in a well flushed water body. However, the data gap for chronic exposure to small quantities of methoprene over the long term, particularly in a poorly flushed medium, prevents conclusions from being drawn about the long term effects of this formulation.

- While some impact on non-target aquatic organisms could be expected, it is clear that the effects of methoprene applications are less harmful than those caused by most mosquitocidal pesticides. Methoprene has longer persistence than *Bti* after application, but also causes greater impact on non-target organisms. Despite this, there is no indication in the literature of permanent disruption to ecosystems after methoprene application.

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APPENDIX A : COMPARISON OF METHOPRENE TO ALTERNATIVE LARVICIDES

"When considering the environmental safety of methoprene use, it is important to compare efficacy with other agents. The efficacy of methoprene in comparison to other mosquitocidal agents has been examined by several researchers in both laboratory and field situations. Methoprene has consistently proved to be one of the most effective insect growth regulators against mosquitoes and is usually more efficacious than biological control agents" *(Glare, 1999)*.

"Methoprene has a broader host range than biological control agents of mosquitoes, such as *Bt*i, *B. sphaericus* and *Lagenidium giganteum.* However, it is far more specific than widely used chemical controls such as temephos. While the list of susceptible insects is extensive for methoprene, many reported susceptible organisms require doses that greatly exceed the field application rate. Several researchers have suggested that methoprene can be specific to Diptera in field situations, which would be likely to include some beneficial dipteran species. The non-target effects observed after methoprene use include some reduction in benthic communities and direct, but low toxicity to fish, however such communities appear to recover quickly" *(Glare, 1999)*.

Bacillus thuringiensis israelensis (Bti)

Bti is a naturally occurring soil bacterium that produces a proteinaceous crystalline inclusions containing toxins that damage the gut of some insects. Bti is approved for control of larval mosquitoes, black flies, and some midges. Bti has been found to be non-toxic to many beneficial predatory insects. "Bti is generally toxic to only nematocerous[30] Diptera, and, as with other Bt larvicides, toxicity to the target group requires both ingestion of the toxic crystals associated with the spores and the appropriate pH conditions in the gut. Laboratory and field studies have shown that Bti is toxic to some larval chironomids, but many factors reduce its toxicity to chironomids in the environment. In black fly control studies conducted in streams, limited short-term effects on insect drift, but not on mortality, have been observed for some Ephemeroptera and Trichoptera" *(Hershey, 1998)*.

Most formulations for use in mosquito control are known by the trade name Vectobac®. Altosid® and *Bti* have been used together for many years and this combination is usually referred to as a Duplex mixture. Duplex has been shown to control all species of mosquitoes. The two control agents are usually applied in ratios of 12:1 to 6:1 of *Bt*i:Altosid®. When a 6:1 ratio is applied at 1 pint/acre, effective control can be maintained for about 10 days *(Glare, 1999)*.

The presence of pollutants, salinity, organic and inorganic particles can all reduce the efficacy of *Bti* efficacy. Pollution apparently results in less *Bti* being ingested, resulting in reduced efficacy. The presence of free chlorine in the water can inhibit or destroy the endotoxin. The presence of soil significantly reduces larval mortality, probably by assisting sedimentation and unavailability of *Bti*. The efficacy of spore-crystal formulations of *Bti* against mosquito larvae (*Cx. quinquefasciatus* and *Ae. Aegypti)* in the laboratory decreased when aqueous environments contained a concentration of soil or clay particles greater than or equal to 0.5 mg/ml. In another study, sludge from soil decreased the effectiveness of *Bti* more than decomposing organic matter, inorganic mud or silica gel. *(Glare, 1998)*.

[30] Nematocera is a suborder of the genus Diptera and includes insects that are small, slender and midge-like in appearance. Mosquitoes are an example of nematocerous Diptera.

It is difficult to compare the toxicity of *Bti* to chemical controls such as methoprene and temephos. *Bt* concentrations are measured in international units, which factor in the potency of each batch and toxicity study results are reported in terms of colony forming units (CFU). The situation is similar for *B. spaericus.* See Table Seven for a list of *Bti* aquatic toxicity endpoint values to aquatic organisms.

Temephos

Temephos is a non-systemic organophosphorus insecticide and the only chemical from this class that is used to control mosquito, midge, and black fly larvae. It is used in lakes, ponds, and wetlands in formulations known by the trade name Abate. The toxicity of temephos to aquatic organisms varies widely with species. Some species for example are more susceptible to methoprene than temephos. Methoprene however, is far more specific than temephos, which effects a much broader range of aquatic insects *(Glare, 1999)*.

Insects appear to develop resistance to temephos more rapidly than to methoprene, another issue in choice of agents for use in vector management. The long residual activity of temephos can make it attractive for specialized uses such as container treatment (which contributes to the development of resistance). This needs to be compared with methoprene, which also has long residual activity in protected (from sunlight) environments *(Glare, 1999)*. A further problem with organophosphates is the likelihood of withdrawal from the market in the future, due to concerns raised by the Food Quality Protection Act such as cumulative toxicity to compounds exhibiting a common mechanism of toxicity.

See Table Seven for a list of temephos aquatic toxicity endpoint values to aquatic organisms.

Bacillus sphaericus

Bacillus sphaericus is a naturally occurring bacterium that is found throughout the world. *Bacillus sphaericus* was initially registered by EPA in 1991 for use against various kinds of mosquito larvae. Most formulations for use in mosquito control are known by the trade name VectoLex®. Mosquito larvae ingest the bacteria, and as with *Bt*i, the toxin disrupts the gut in the mosquito by binding to receptor cells present in insects, but not in mammals. VectoLex® CG and WDG are registered *B. sphaericus* products, and are effective for approximately one to four weeks after application.

Poly Ethoxylated Alcohols and Monomolecular Films

Monomolecular films (MMF) or poly ethoxylated alcohols (POE) are manufactured by the reaction of alcohols with ethylene oxide. When these materials are applied to bodies of water they form a thin film on the surface. This film reduces the surface tension of the water and mosquito larvae and pupae are unable to attach to the surface, which they must do in order to breathe. This film may also block their breathing tubes. Films may remain active for typically 10-14 days on standing water, and have been used in the United States in floodwaters, brackish waters, and ponds. They may be used along with other mosquito control measures in an IPM program. They are also known under the trade names Arosurf® MSF and Agnique® MMF. At application rates up to 0.5 Gal/Surface Acre, Agnique® MMF Mosquito Larvicide and Pupicide is registered for use in semi-permanent or permanent fresh potable and irrigation water. It is also registered for use in salt water habitats with no, low, moderate or high concentrations of emergent or surface vegetation. Examples of these systems include: salt marshes, ponds, storm water retention/detention basins, roadside ditches, grassy swales, potholes, fields, reservoirs, irrigated croplands, etc..

Other use sites include semi-permanent or permanent polluted water habitats containing no, low, moderate, or high concentration of algal mats, emergent or surface vegetation and/or organic/inorganic debris.

Table Eight Aquatic Toxicity of Mosquito Larvicides (Hicks, 2001)				
Active Ingredient	**Warm water fish LC_{50} (Median Lethal Concentration)**	**Cold water fish LC_{50}**	**Estuarine and Marine Toxicity**	**Freshwater Invertebrates**
Bti [1]	Bluegill Sunfish; Aqueous $LC_{50;}$ 8.9×10^9 to 1.6×10^{10} colony forming units per liter (cfu/l) [1] Oral $LC_{50} > 4.3 \times 10^9$ to 1.3×10^{10} cfu/gram food [1]	Trout; Aqueous $LC_{50;}$ $> 8.7 \times 10^9$ to $> 1.4 \times 10^{10}$ cfu/l [1] Oral $LC_{50} > 5.3 \times 10^9$ to 1.7×10^{10} cfu/gram food [1]	Grass shrimp; No Observable Effect Level (NOEL) $> 2 \times 10^{10}$ cfu/g food [1] NOEL $> 4.2 \times 10^{10}$ cfu/g food [1] Sheepshead minnow; NOEL $> 2 \times 10^{10}$ cfu/g food [1] $LC_{50} > 7.2 \times 10^9$ cfu/g food [1] Oral $LC_{50} > 2 \times 10^{10}$ cfu/g [1] Copepod NOEL = 50 mg/kg (sediment) [1]	Daphnia 21 Day (EC_{50}) Median Effective Concentration = 5,000 - 50,000 parts per billion (ppb) = ug/L [1]
B.sphaericus [2]	ND = No Data	ND	ND	ND
Methoprene [3]	Bluegill sunfish: 96hr LC_{50} 1,520ppb [3] 96 hr TL_{50} (median threshold limit) = 4,600 ppb (static) [2] $LC_{50} > 370$ ppb [3] Channel catfish: $TL_{50} > 100{,}000$ ppb (static) [54] Fathead minnow: LEL (Lowest Effective Level) = 84 ppb [22b] NOEL = 48 ppb [22b]	Rainbow trout: 96 hr $LC_{50} > 50{,}000$ ppb [3] Juvenile Rainbow trout: $LC_{50} = 106{,}000$ ppb [54] $LC_{50} = 760$ ppb [22b] $LC_{50} = 106{,}000$ [54] Trout: $TL_{50} = 4{,}400$ ppb (static) [54] $TL_{50} = 106{,}000$ ppb (static aerated) [54] Coho salmon LC 50 = 86,000 ppb [54]	Mud crab: ↓ gametes in @ 1,300 ppb [3] Adult grass shrimp: Slightly toxic [3] not acutely toxic [41] Juvenile grass shrimp and larval mud-crabs: Very highly toxic [1] not acutely toxic Gammarus aequicauda: 96 hr $LC_{50} = 2{,}150$ ppb (females) [54, 22d] 96 hr $LC_{50} = 1{,}950$ ppb (males) [54, 22d] Mysid Shrimp: 96 hr $LC_{50} = 110$ ppb [22b] 28 day MATC = > 98 ppb [22b] Oyster (larvae): 48 hr $LC_{50} = 247$ ppb [22b] Oyster shell deposition 96 hr = 1,400 ppb [22b]	Daphnia; 48 hr EC_{50} 89 ppb [3] 42 day MATC 27 - 51 ppb [3] 48 hr $EC_{50} = 360$ ppb [22b] 42 day MATC 51 ppb [22b]

Table Eight Aquatic Toxicity of Mosquito Larvicides (Hicks, 2001)				
Active Ingredient	**Warm water fish LC_{50} (Median Lethal Concentration)**	**Cold water fish LC_{50}**	**Estuarine and Marine Toxicity**	**Freshwater Invertebrates**
POE MMF [5]	Bluegill sunfish: LC_{50} = 290,000 ppb [34]	Rainbow trout: LC_{50} = 98,000 ppb [34]		*Daphnia*: LC_{50} = 1,900 ppb [34]
Temephos (Abate) [4] EC	Bluegill Sunfish; 96 hr LC_{50} = 21,800 ppb Technical Grade Active Ingredient (TGAI) [4, 33] 96 hr LC_{50} = 1,140 ppb Emulsifiable concentrate 43% (EC)[4, 33] Fathead minnow: 31,100 ppb [33] Channel catfish: 10,000 ppb [33] 3,230 (EC 46%) [33] Largemouth bass: 1,440 ppb (EC 46%) [33]	Rainbow trout; 96 hr LC_{50} = 3,490 ppb (TGAI) [4, 33] 96 hr LC_{50} = 580 ppb (EC) [4] 160 ppb (EC) [33] Cut throat trout: 1,279 ppb [33] Brook trout 12,800 ppb [33] 5,000 ppb (WP 50%) [33] Lake trout 3,650 ppb [33] Coho salmon 350 ppb (EC 46%) [33] Atlantic salmon 21,000 ppb [33] 6,700 ppb (EC 46%) [33]	Eastern oyster; 96 hr EC_{50} = 220 ppb (TGAI) [4] 96 hr EC_{50} = 170 ppb (EC) [4] Pink Shrimp; 48hr EC_{50}= 5.3 ppb (EC) [4] *Gammarus lacustris* 80 ppb [33]	Daphnia 48 hr LC_{50} = 0.011 ppb (EC) [4] 48 hr LC_{50} = 0.54 ppb Granular %5 (G) [4] Scud; 48 hr LC_{50} = 820 ppb (TGAI) [4] Stonefly; 48 hr LC_{50} = 10 ppb (TGAI) [4]

[1] EPA (1998) R.E.D. Bacillus thuringiensis variety *israelensis* (Bti).
[2] Bacillus sphaericus.
[3] EPA (1991) R.E.D. Methoprene.
[4] EPA (1999) ERED Reregistration Chapter for Temephos.
[22b] Sandoz (1996) Submission of Environmental Toxicity and Release Data to EPA.
[22d] Grandoni, L., Bettini, S. and Majors, G. 1976. *Toxicity of Altosid to the Crustacean:* Gammarus aequicauda. Mosquito News, Vol. 36(3):294-297.
[33] Hazardous Substances Data Base (2001) for Temephos: (http://toxnet.nlm.nih.gov)
[41] Wellmark (2001) Comments on March 5,2001 Maine draft report by Hicks, Lebelle:
[54] Vershcueren, K. Handbook of Environmental Data on Organic Chemicals. 2nd Ed. Van Nostrand Reinhold Press, NY, 1983.

APPENDIX B: JUNE 2001 EPA UPDATE OF THE MARCH 1991 METHOPRENE R.E.D. FACTSHEET

http://www.epa.gov/pesticides/biopesticides/factsheets/fs105401.pdf